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14. ABSTRACT

Chronic migraine is a disabling disorder that affects millions of individuals worldwide, and may result from traumatic brain injury. The purpose of this study is to use rodent models of basic migraine mechanisms to characterize new potential treatments for chronic migraine. The scope of the research is to investigate multiple novel potential drug treatments on migraine-related brain excitability, pain-sensing mechanisms, and behavior. The major findings of the research in the first year of the project are that the medication amiloride inhibits the phenomenon of cortical spreading depression, a wave of activity that is believed to underlie the migraine aura. Amiloride also prevents the development chronic migraine-related hyperalgesia in mice. These results support the clinical investigation of amiloride and related drugs as treatments for chronic migraine. We have also found that the drug memantine inhibits cortical spreading depression, but acute treatment with memantine does not inhibit nociceptive signaling in the brainstem. These results are consistent with the potential efficacy of memantine as a preventive therapy, but not as an acute therapy for migraine. We have also found that delta opioid receptor agonists inhibit both cortical spreading depression and migraine related pain behaviors, also supporting the clinical investigation of these compounds. Overall, we have made significant progress in the characterization of novel potential therapies for chronic migraine.

15. SUBJECT TERMS
Migraine, Therapy, Amiloride, Memantine, Canrenone

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INTRODUCTION:

This project investigates basic mechanisms of migraine, a disorder that afflicts hundreds of millions of individuals worldwide. Chronic migraine, defined as more than 15 days of headache per month, affects 4% of the population and is a particularly disabling condition. Traumatic brain injury is a common trigger for common migraine.

BODY:

The progress on each of the originally proposed tasks is summarized below.

Task 1. Characterization of the effects of memantine on migraine models

a. Effects of memantine on CSD - We have initial studies (n=4) that indicate that chronic treatment with memantine reduces the numbers of CSD events evoked by chronic application of KCl. We are currently performing additional experiments to increase the n-number and determine statistical significance.

b. Effects of memantine on trigeminovascular nociceptive activation. Studies by the Goadsby group indicate that memantine *does not* inhibit trigeminovascular nociceptive activation in mice.

c. Effects of memantine on acute and chronic nitroglycerin-evoked hyperalgesia in mice: Studies are ongoing.

Conclusions - The results obtained thus far suggest that while memantine inhibits migraine-related cortical excitability, it does not inhibit trigeminovascular nociceptive activation. These results indicate that memantine may be effective as a preventive therapy in the treatment of migraine, but may not be effective as an acute therapy. Further conclusions will be based on completion of the studies in 1a. and 1c.

Task 2. Characterization of the effects of amiloride on migraine models

a. Effects of amiloride on CSD - Our studies indicate that acute application of amiloride inhibits CSD. We are currently performing studies to determine if chronic administration of amiloride has a similar effect.

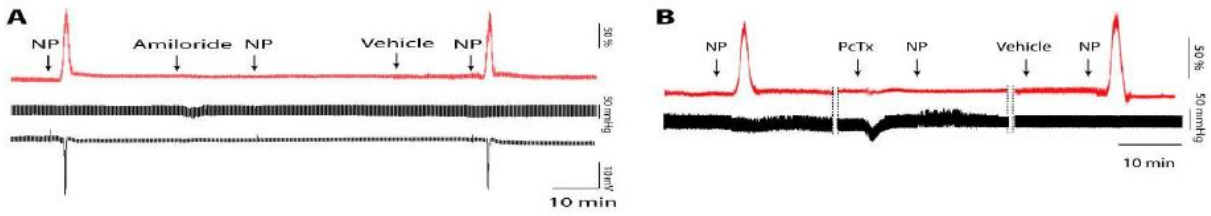

Figure 1 The effects of amiloride on cortical spreading depression
(A) Regional cerebral blood flow (top), blood pressure (middle) and DC shift (bottom) recordings from a sample cortical spreading depression (CSD) trace. Amiloride at 10mgkg-1 significantly inhibited needle prick-induced (NP; 6 of 8) CSDs when compared to vehicle controls (P = 0.007, Fisher's exact test), while having no significant effect on blood pressure. The lower dose of 5mgkg-1 had no significant effect on CSD propagation (P = 1.00, Fisher's exact test), inhibiting only 1 of 8.(B) Regional cerebral blood flow (top) andblood pressure recordings from a sample CSD trace. Psalmotoxin (PcTx), the specific ASIC1a blocker significantly inhibited NP induced CSDs (5 of 6) when compared to vehicle control (P = 0.015, Fisher's exact test), but resulted in adverse blood pressure effects.

b.Effects of amiloride on trigeminovascular nociceptive activation

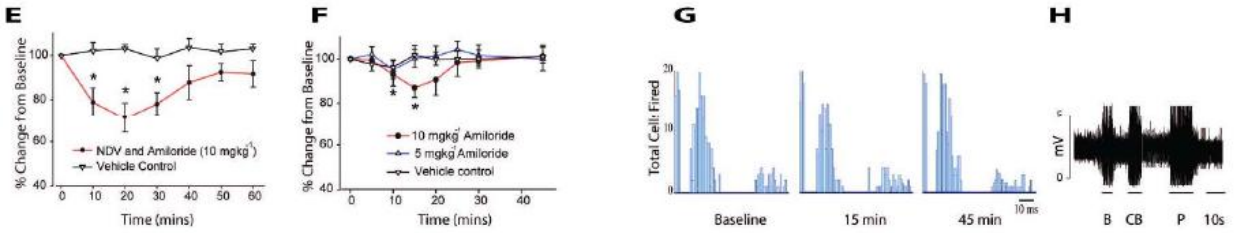

Figure 2. Effects of amiloride on trigeminovascular activation
(E) Amiloride 10mgkg-1 inhibited neurogenic vasodilation (F6, 42 = 5.1, P <0.001; n = 8) of the middle meningeal artery (MMA), maximally by 29% at 20 minutes post drug (t7 = 4.6, P < 0.002). (F) Effect of amiloride on trigeminal nucleus caudalis neuronal responses to stimulation of the
dura mater surrounding the MMA. Amiloride at 10, but not 5mgkg-1 inhibited neuronal responses (F7,49 = 3.15, P < 0.01; n = 8), significantly decreasing A-fiber responses at 10 and 15 minutes post drug when compared to baseline and vehicle controls, maximally by 16% returning to baseline levels after 20 minutes. (G) Original post-stimulus histogram showing decreased A-fiber response 15 minutes post amiloride returning to baseline levels after 45 minutes. (H) Original example of second order trigeminal neuronal responses to facial receptive field stimulation including light brush (B), corneal light brush (CB) and noxious pinch (P). Amiloride had no effect on any aspect of receptive field studied.

c. Effects of amiloride on acute and chronic nitroglycerin-evoked hyperalgesia in mice
We have found that amiloride inhibits the development of chronic basal hyperalgesia in response to chronic administration of nitroglycerin. However, amiloride given acutely does not inhibit acute nitroglycerin-evoked hyperalgesia. This is in contrast to sumatriptan, which inhibits acute nitroglycerin-evoked hyperalgesia but has no effect on the development of chronic basal hyperalgesia.

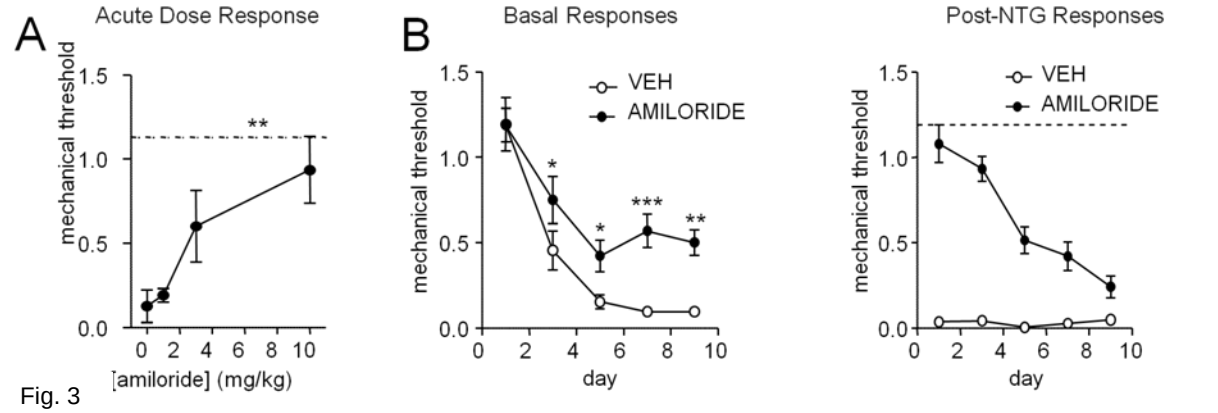

Fig. 3
Amiloride attenuated NTG-induced mechanical hypersensitivity (A) Amiloride dose-dependently attenuated mechanical hyperalgesia in an acute model of NTG (10 mg/kg IP) pain. **(B)** Amiloride (10 mg/kg IP) attenuated the basal hypersensitivity observed following chronic intermittent nitroglycerin treatment. **(C)** Tolerance was observed to the acute pain-relieving effects of amiloride. Dashed lines indicate basal responses on day 1 (naïve). n=8/group; *p<0.05, **p<0.01, ***p<0.001.

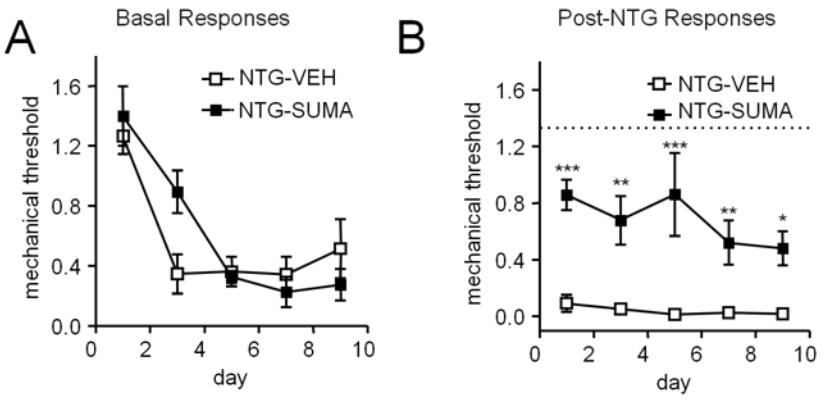

Fig. 4

Acute NTG-evoked hyperalgesia is alleviated by sumatriptan. (A) Treatment with sumatriptan (600 µg/kg IP) did not affect the basal hypersensitivity induced by nitroglycerin (10 mg/kg IP) treatment. (B) Sumatriptan significantly attenuated nitroglycerin-evoked hyperalgesia (post-NTG responses; n=5-6/group; *p<0.05, **p<0.01, ***p<0.001. Dashed line indicates basal responses on Day 1 (naïve).

Conclusions These results show that amiloride inhibits spreading depression, trigemino-vascular nociception, and chronic but not acute nitroglycerin-evoked hyperalgesia. Amiloride's effects on multiple migraine mechanisms supports formal investigation as a migraine preventive therapy.

Task 3. Characterization of the effects of canrenone on migraine models
We have not yet begun formal experiments with canrenone

Technical accomplishments
In order to enhance the information obtained from the cortical spreading depression studies and allow for more rapid and efficient drug screening studies, we have developed a high resolution system for optical intrinsic signal imaging through a minimally thinned skull in mice, in conjunction with highly synchronized electrophysiological recording as well as recording of blood pressure, pulse, oxygen saturation, and respiration. This system will allow us to gain even more information about the effects of medications on cortical excitability and vascular function.

KEY RESEARCH ACCOMPLISHMENTS:

- Acute dosing of amiloride inhibits cortical spreading depression
- Acute dosing of amiloride inhibits multiple trigeminovascular migraine-related mechanisms
- Chronic dosing of amiloride inhibits chronic migraine-related hyperalgesia but not acute amiloride does not inhibit acute hyperalgesia
- Development of a novel high resolution imaging and physiolological monitoring system for recording cortical spreading depression in minimally invasive mouse preparation.

REPORTABLE OUTCOMES: Provide a list of reportable outcomes that have resulted from this research to include:

Manuscripts:

1. Holland, P., Akerman, S., Andreaou, A., Karsan, N., Wemmie, J., and Goadsby, P. Acid-sensing ion channel-1: a novel therapeutic target for migraine with aura. Annals of Neurology, In Press, 2012.

2. Pradhan, A., Smith, M., Zyuzin, J., Charles, A. Delta opioid receptor agonists Inhibit migraine-related pain, aversive State, and cortical spreading depression. Submitted, 2012.

Abstracts/presentations:

Pradhan, A., McGuire, B., and Charles, A. Characterization of a novel model for chronic migraine. Poster presentation for European Migraine Foundation/Migraine Trust Scientific Meeting. London, September 2012. Selected (4/30 in session) for oral presentation.

Hoffmann, J.; Park, J.W.; Storer, R.J.; Goadsby, P.J. Magnesium and memantine do not inhibit nociceptive neuronal activity in the trigeminocervical complex of the rat. Poster presentation for European Migraine Foundation/Migraine Trust Scientific Meeting. London, September 2012.

Funding applied for based on work supported by this award

NIH RO1 - Andrew Charles and Peter Goadsby, Co-PI's, "Novel Migraine Mechanisms as Targets for Therapy"

CONCLUSION: In the first year of this project we have made significant progress in the investigation of the effects of potential treatments on basic mechanisms of migraine. We have provided solid pre-clinical evidence for the potential efficacy of amiloride as a preventive therapy for migraine that justifies further investigation in clinical trials. We have initial similar information regarding memantine. We have enhanced our system for high resolution imaging and physiological monitoring of cortical spreading depression. We are poised to make further progress toward a better understanding of basic migraine mechanisms and to move forward with new potential therapies.

REFERENCES: None

APPENDICES: None